Kidney International, Vol. 68, Supplement 99 (2005), pp. S46–S51

Role of hypoxia in the pathogenesis of renal disease

KAI-UWE ECKARDT, WANJA M. BERNHARDT, ALEXANDER WEIDEMANN, CHRISTINA WARNECKE, CHRISTIAN ROSENBERGER, MICHAEL S. WIESENER, and CARSTEN WILLAM

Department of Nephrology and Hypertension, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany; and Department of Nephrology and Medical Intensive Care, Charité, Berlin, Germany

Role of hypoxia in the pathogenesis of renal disease. The kidney shows a remarkable discrepancy between blood supply and oxygenation. Despite high blood flow and oxygen delivery, oxygen tensions in the kidney are comparatively low, in particular in the renal medulla. The reason for this lies in the parallel arrangement of arterial and venous preglomerular and postglomerular vessels, which allows oxygen to pass from arterioles into the postcapillary venous system via shunt diffusion. The limitation in renal tissue oxygen supply renders the kidney susceptible to hypoxia and has long been recognized as an important factor in the pathogenesis of acute renal injury. In recent years, evidence has accumulated that hypoxia does also play a significant role in the pathogenesis and progression of chronic renal disease, because different types of kidney disease are usually associated with a rarefication of postglomerular capillaries. In both acute and chonic diseases, tissue hypoxia does not only imply the risk of energy deprivation but also induces regulatory mechanisms and has a profound influence on gene expression. In particular, the transcription factor hypoxia inducible factor (HIF) is involved in cellular regulation of angiogenesis, vasotone, glucose metabolism, and cell death and survival decisions. HIF has been shown to be activated in renal disease and presumably plays a major role in protective responses to oxygen deprivation. Recent insights into the regulation of HIF increase our understanding of the role of hypoxia in disease progression and open new options to improve hypoxia tolerance and to induce nephroprotection.

Oxygenation of the kidney is characterized by a remarkable paradox. On the one hand, in relation to their weight, the kidneys are the very best perfused organs of the organism, receiving an overall oxygen supply of more than 80 mL/min × 100 g weight, of which less than 10% is consumed during its passage through the kidneys [1]. On the other hand, tissue oxygen tensions in the renal parenchyma are lower than in most other organs and much below those measured in the renal vein [2]. In particular, the renal medulla is considered one of the sites in the body with the lowest oxygen tensions. The explanation for the discrepancy between high oxygen supply and low tissue oxygen tensions of the kidney lies in the unique architecture of the renal vasculature. In both the cortex and the medulla, branches of the renal arteries and veins run strictly parallel and in close contact with each other over long distances. This parallel arrangement allows oxygen to diffuse from the arterial system into the venous system before it has entered the capillary bed [3, 4]. Thus oxygen is subjected to a countercurrent exchange comparable with urea, and this mechanism is particularly relevant in the renal medulla, where it leads to oxygen tensions below 10 mm Hg [2]. However, the countercurrent exchange of oxygen also occurs in preglomerular arteries of the renal cortex, where oxygen tensions are about 30 mm Hg with marked variability [2, 4].

At the same time, most tubular segments have a very limited capacity for anaerobic energy generation and are thus dependent on oxygen to maintain active transtubular reabsorption of solutes, in particular sodium. The combination of limited tissue oxygen supply and high oxygen demand is considered the main reason for the susceptibility of the kidney to acute ischemic injury [5].

In recent years, increasing evidence has accumulated, indicating that a reduction in renal oxygen tensions also plays an important role in the progression of chronic kidney disease [6]. The impact of hypoxia, respectively ischemia, on the progression of renal disease can be summarized through three main points. First, it has become clear that the peritubular capillary bed in the kidney, which provides the structural basis for adequate oxygen delivery to tubular cells, is a rather dynamic structure and that chronic diseases of the kidney are associated with a rapid decline in capillary density. Second, as a consequence of capillary loss and capillary hypoperfusion, tissue oxygen tensions usually decline in a diseased kidney (Fig. 1; Table 1). Third, low oxygen tensions may not only impair energy generation but act as a regulator of cellular functions and as a specific stimulus for the induction of certain genes. Altogether, these findings suggest that hypoxia is an important factor in the progression of kidney disease. Unravelling the underlying molecular and cellular mechanisms is likely to increase our understanding of

Key words: hypoxia, ischemia, renal injury, gene expression, hypoxia inducible factor, HIF.

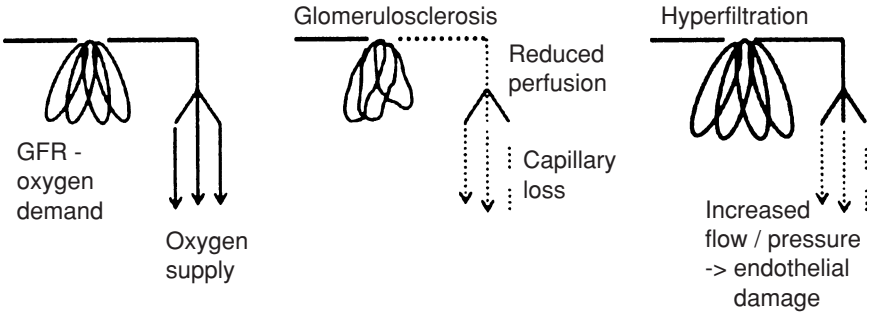

Fig. 1. Schematic presentation of peritubular capillary perfusion in health and disease. While the amount of filtered sodium is the main determinant of renal oxygen consumption, the flow through capillaries arising from the glomerular vasa efferentia determines oxygen supply. When glomerular desease impairs glomerular perfusion, this will automatically impair peritubular perfusion. In addition, loss of peritubular capillaries impairs peritubular perfusion and increases diffusion distances. In contrast, glomeruli undergoing compensatory hypertrophy will be associated with an increase in glomerular and peritubular perfusion, which might also cause microvascular damage.

Table 1. Factors possibly contributing to renal hypoxia in chronic kidney disease

-Anemia
-Renal artery stenosis
-Loss of peritubular capillaries
-Impaired peritubular blood flow due to glomerular hypoperfusion and/or an imbalance of vasoactive substances
-Increased diffusion barriers due to tubulointerstitial fibrosis

the progression of renal injury and may open new therapeutic avenues.

DECREASED PERITUBULAR CAPILLARY DENSITY

Approximately 10 years ago, Bohle and coworkers observed in human renal biopsies that the number of peritubular capillaries declines with progressive loss of renal function [7]. More recently, a loss of peritubular capillaries has been reproduced in different animal models, including experimental glomerulonephritis [8], the remnant kidney model [9], ureteral obstruction [10], ischemia/reperfusion [11], and renal artery stenosis [12]. Interestingly, the decline in capillary density occurs rapidly within days after the initial insult and persists for weeks, in parallel with an increase in peritubular matrix deposition. There is evidence for programmed cell death of endothelial cells by apoptosis [8] and for an imbalance of angiogenesis regulators, with decreased expression of pro-angiogenic molecules, such as vascular endothelial growth factor, and induction of anti-angiogenic molecules, such as thrombospondin [9, 13–15]. Moreover, the comparison of different mouse strains revealed that genetic predisposition plays an important role in the effect of nephron loss on capillary density [16].

However, the initial signals that trigger the regressive response of the vasculature in different disease models remain to be elucidated. Obviously inflammatory mediators and oxidative stress [12] could play an important role, but hemodynamic factors may also be relevant. A unique feature of renal oxygenation is the tight link between the glomerular filtration rate, the main determinant of renal function and peritubular oxygen supply. Because peritubular capillaries are derived from the vasa efferentia of the glomeruli, any disturbance of glomerular blood flow resulting from glomerular capillary obstruction or sclerosis will automatically impair peritubular perfusion. In addition, a compensatory increase in perfusion of other glomeruli may increase flow and pressure in the peritubular capillary network and thereby cause microvascular injury, which induces capillary regression.

Regardless of what the precise mechanisms may be, Kang and coworkers have demonstrated the functional significance of the regression of the capillary network. They showed that endothelial proliferation induced by vascular endothelial growth factor treatment in the rat remnant kidney model results in improved renal function and lower mortality rates [17].

EVIDENCE FOR TISSUE HYPOXIA IN CHRONIC KIDNEY DISEASE

A priori it is unclear whether impaired renal perfusion affects tissue oxygenation. Because glomerular perfusion determines both oxygen supply (through peritubular capillaries) and oxygen consumption (which is directly related to the filtered and reabsorbed sodium load), the effect of changes in glomerular perfusion on the net balance of renal oxygen tensions is difficult to predict. In fact, renal artery stenosis was shown to be an infrequent and mild stimulus for erythropoietin (EPO) formation, which is normally produced in inverse relationship to peritubular cortical oxygen tension [18].

Unfortunately, very limited data are available about the profiles of oxygen tensions in kidneys with different forms of chronic disease. Several investigators used immunostaining techniques with the bioreductive agent pimonidazole, which is known to accumulate in hypoxic tissues and has been used as a radiosensitizer to treat malignant tumors [19]. They reported increased staining in an experimental glomerulonephritis model [20], puromycin aminonucleoside nephropathy [21], the remnant

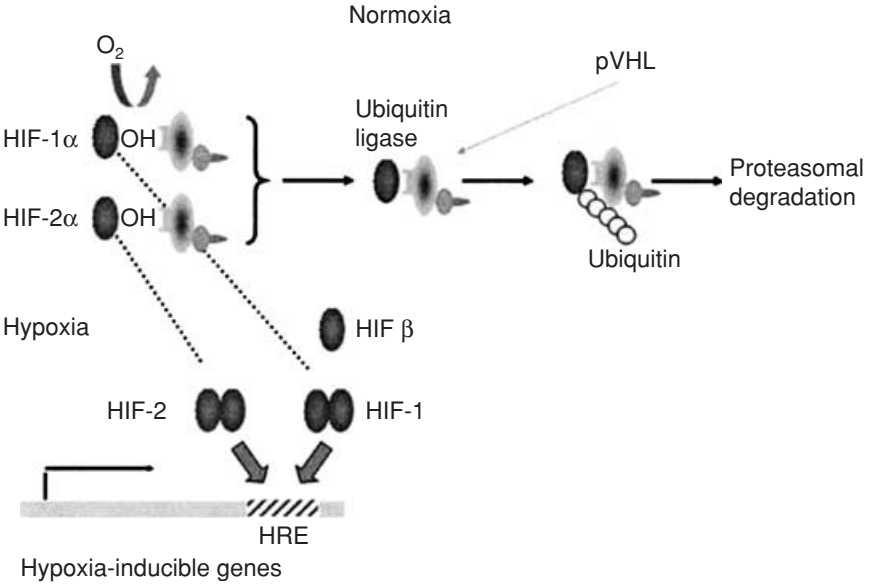

Fig. 2. Schematic view of hypoxia-responsive gene expression mediated by HIF. HIF is a heterodimer, composed of an oxygen-regulated α-subunit (HIF-1α or HIF-2α) and a constitutive β-subunit. In the presence of oxygen-specific prolyl residues of HIFα are hydroxylated, and hydroxylated HIF can then bind to an ubiquitin-ligase complex, which targets HIF for destruction through the ubiquitin proteasome pathway. When insufficient oxygen is available for the hydroxylation reactions (i.e., hypoxia), HIFα does not enter the destruction pathway and, together with its constitutive partner HIFβ, can bind to hypoxia-response elements in genes that are inducible under hypoxic conditions. An additional hydroxylation reaction regulates HIF transcriptional activity (not shown).

kidney model [21, 22], as well as in an ischemia/reperfusion injury model several weeks after recovery [23]. Careful analysis of the time course of changes in a progressive model of rat glomerulonephritis suggested that hypoperfusion of peritubular capillaries induces chronic hypoxia before progression of tubulointerstitial fibrosis [20].

A limitation of the use of pimonidazole is that immunohistochemistry needed to visualize binding of the dye is a non-quantitative method, and that the relationship between the staining intensity and tissue oxygen tensions may be altered by factors that are difficult to control. Noteworthy, a single study, in which oxygen tensions were actually measured in the remnant kidney model using oxygen electrodes, showed higher rather than lower oxygen tensions in the diseased kidneys [24]. The authors suggested that this increase in renal oxygenation explains an "inadequately" low erythropoietin production despite moderate anemia, which occurs in this model.

Considering these different findings, it appears likely that changes in renal oxygen tensions in chronic kidney disease are not uniform, but depend, for example, on the type of renal disease, the time course of injury, and its progression. Additional recordings of tissue oxygen tensions in various models to verify such influences would certainly be desirable. In addition, information about oxygen tensions in human kidneys would also be of significant interest. Blood oxygen level–dependent magnetic resonance imaging has been introduced as a technique that can be used in humans to visualize changes in renal oxygenation [25]. Blood oxygen level –dependent magnetic resonance imaging has been used to demonstrate changes in oxygenation in an animal model of diabetic nephropathy [26], but has not yet been systematically applied to patients with chronic kidney disease.

HYPOXIA INDUCIBLE FACTOR EXPRESSION AND THE EFFECTS OF LOW OXYGEN TENSIONS ON RENAL CELL FUNCTION

Unless oxygen supply is insufficient for the generation of adenosine triphosphate, the actual level of cellular oxygen tensions has long not been considered to be an important variable. In recent years, however, this paradigm was completely revised. It has become clear that variations in oxygen tensions continuously regulate many aspects of cellular functions.

An important aspect of this adaptation is oxygen-dependent gene regulation mediated by the hypoxia-inducible transcription factors (HIF). HIFs are heterodimers of an oxygen- regulated α-subunit and a constitutive β-subunit. Two isoforms of the α-subunit have been in the focus of interest, which are structurally related and regulated in a similar fashion (Fig. 2).

Under normoxic, baseline conditions, we have not detected these transcription factors in any region of the kidney, despite the chronically low oxygen tensions, in particular in the renal medulla [27]. Exposing animals to systemic hypoxia, however, revealed a marked capacity of virtually all renal cells to induce a transcriptional response through HIF-activation. Interestingly, HIF-1α and HIF-2α are differentially expressed. HIF-1α accumulates in tubular epithelial cells of most nephron segments, while the expression HIF-2α is found in peritubular endothelial cells and fibroblasts as well as in glomerular cells [27]. Acute renal hypoxia due to ischemia/reperfusion [21, 27], renal infarction [28], or radiocontrast application [29] (Fig. 3) also induces HIF. Data on HIF expression in chronic renal disease are yet limited. To circumvent the technical problems of directly visualizing HIF, which is rapidly degraded on reoxygenation, and to obtain additional evidence for its functional significance, Tanaka

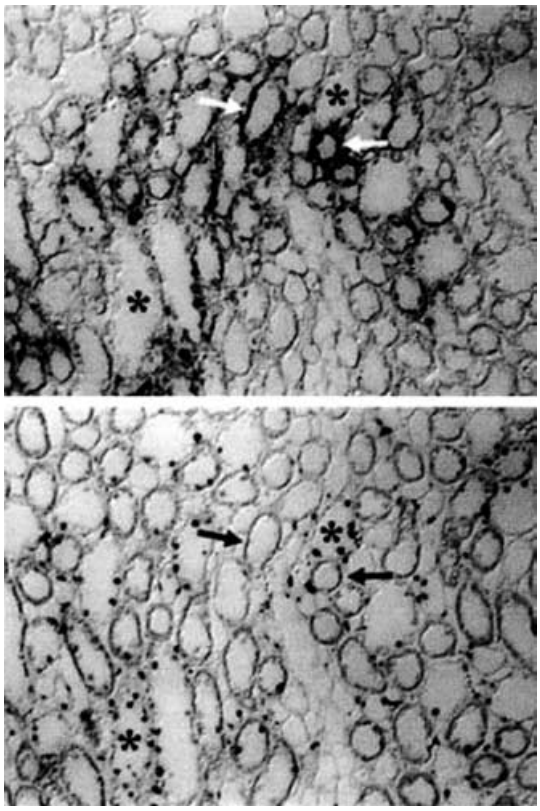

Fig. 3. Follow-up sections of the outer medulla in a rat kidney model of radiocontrast nephropathy [29] stained for the bioreductive marker pimonidazole (upper panel) and for HIF-1α (lower panel). Note marked accumulation of HIF-1α in tubules in the immediate vicinity of tubules that stain most strongly for pimonidazole (arrows). These findings suggest that HIF accumulates in hypoxic cells, but that the ability to induce HIF declines under very severe hypoxia.

and coworkers have recently generated transgenic rats expressing a reporter gene driven by the HIF DNA-binding site, the "hypoxia response element." Using two different models, puromycin aminonucleoside nephropathy and the remnant kidney model, they found transgene activation and thus indirect evidence for induction of the HIF pathway [21].

HIF is considered a master regulator of gene expression in response to hypoxia. Its target genes include erythropoietin, angiogenic growth factors, heme-oxygenase 1, glucose transporter 1, and almost all glycolytic enzymes [30]. In addition, HIF target genes are also involved in cell survival decisions, including apoptosis, and both pro- and antiapoptotic effects have been described [31, 32]. The majority of cellular effects of HIF induction are likely to confer adaptation and protection against hypoxic injury, and HIF induction is therefore a potential strategy for nephroprotection (see below). On the other hand, under certain circumstances, genes induced by hypoxia may also be maladaptive and, in particular, may promote renal fibrosis. Thus, hypoxia was found to induce collagen messenger RNA expression and induce tissue inhibitors of metalloproteinases [33]. Transforming growth factor β can be directly induced by hypoxia [34] and, in addition, Ctgf, a downstream mediator of profibrotic cytokines, including transforming growth factor β, can be induced by hypoxia through HIF activation [35]. Hypoxia also promotes the transdifferentiation of proximal tubular cells into myofibroblasts [33, 36].

Table 2. Theoretical options to reduce renal tissue hypoxia or improve hypoxia-adaptation

-Increasing renal oxygen delivery, for example, by increasing the hemoglobin concentration
-Percutaneous transluminal angioplasty or surgical revascularization procedures
-Improving microvascular density by stimulating angiogenesis or avoiding capillary regression
-Improving the regional balance between oxygen delivery and consumption, for example, by inhibiting the renin angiotensin system
-Antifibrotic strategies
-Activating HIF, for example, by prolyl hydroxylase inhibitors

Another aspect in how hypoxia may indirectly promote renal injury is an increase in blood pressure. Exposure of rats to systemic hypoxia leads to a significant rise in blood pressure that persists after return-to-normal ambient oxygen tensions [37]. This rise is associated with microvascular endothelial changes, subtle tubulointerstitial injury, inflammation, and interstitial cell proliferation. Johnson et al have postulated such microvascular changes as a uniform pathway for the development of salt-sensitive hypertension [38].

An additional role of hypoxia may be its effect on maintenance and recruitment of adult renal stem cells. A recent report indicates that the renal medulla serves as a niche for adult renal stem cells, which resume proliferation and are recruited into different zones of the kidney after tubular damage [39]. Hypoxia modulates the phenotype of isolated papillary cells [39], and HIF is believed to play a central role in stem cell responses in different organs [40, 41].

TARGETING RENAL HYPOXIA AS A THERAPEUTIC AIM

Given the increasing, albeit yet fragmentary, evidence for an important role of hypoxia in the pathogenesis and progression of renal disease, attempts to avoid a reduction in renal tissue oxygen tensions and/or manipulating and supporting the adaptive reactions could help to preserve renal structure and function (Table 2).

In general, improving renal oxygenation could be achieved by enhancing systemic oxygen transport or regional oxygen supply. The question as to whether correcting renal anemia with recombinant EPO has an impact on the progression of renal disease is still under debate (see also Rossert et al, this issue). Although the majority of

investigations performed so far have not revealed a relevant beneficial or adverse effect of anemia correction on the decline of glomerular filtration rate, a recent study suggested that anemia correction may retard the time to dialysis in patients with advanced chronic kidney disease [42].

Improving regional oxygen supply in the kidney independent of blood oxygen transport capacity could hypothetically be achieved with angiogenesis stimulators that promote peritubular capillary formation. Although such approaches have been successful in experimental models, as discussed, the complexity of angiogenesis modulation does not seem to render this approach realistic in the near future.

On the other hand, some of the beneficial effects of inhibiting the renin angiotensin system could be due to an improvement of renal oxygenation. Relieving efferent arteriolar tone by angiotensin II inhibition will reduce the filtered load and increase peritubular perfusion, thereby increasing the ratio between oxygen supply and demand. Indeed renin-angiotensin-aldosterone system inhibition increases renal cortical oxygen tensions acutely [43] and ameliorates tubular hypoxia in the remnant kidney model [22]. In humans this mechanism has been indirectly observed as an inhibitory effect of angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists on EPO production and hemoglobin levels [44]. However, the extent to which the protective effect of renin-angiotensin-aldosterone system inhibition can be attributed to improved renal oxygenation remains unclear.

The use of EPO also has to be considered among strategies aiming to induce tissue- and cytoprotection. There is increasing evidence that in addition to its main effect as a growth and survival factor of red cell precursors, EPO has paracrine effects mediated through EPO receptors in various organs that confer protection against hypoxic injury [45]. In fact, cell and tissue protection, independent of hemoglobin levels, has been demonstrated in isolated renal cells [46], as well as in the ischemia reperfusion model [47, 48] and in models of chronic renal injury [49]. Whether these findings are of any relevance under the conditions of EPO therapy usually applied for anemia correction in patients with chronic kidney disease, however, remains to be determined. In fact, increasing hemoglobin levels with EPO therapy is likely to downregulate residual endogenous EPO production, so that the local EPO concentration could theoretically even decrease under exogenous hormone therapy.

Increased recognition of the molecular mechanisms regulating HIF has recently opened new options for hypoxia protection that are independent of the delivery of a specific gene product. In the presence of oxygen, the α-subunits of HIF (HIF-1α and HIF-2α) are rapidly degraded through the ubiquitin proteasome pathway. This degradation is initiated by the hydroxylation of two prolyl residues of HIFα, which requires oxygen as a substrate. Only hydroxylated HIF can bind to an ubiquitin-ligase complex, which targets HIF for proteasomal destruction [50, 51]. As a consequence, inhibitors of the prolyl hydroxylases, those enzymes which hydroxylate HIF, inhibit HIF degradation and mimic the effect of hypoxia in the presence of oxygen [50, 52]. Such inhibitors have been found to upregulate HIF in the kidney in vivo and induce angiogenesis in an experimental model [53]. Cobalt chloride, which can also stimulate HIF by interfering with its degradation, was shown to confer protection in an acute renal failure model [54] and a rat glomerulonephritis model [55]. Further proof of the protective effect of HIF was recently provided in a rat ischemia model in which gene transfer of a constitutively active HIF conferred medullary protection [56].

Whether similar protective effects can be achieved in chronic disease models is not yet clear. Interestingly, however, prolyl hydroxylase inhibitors are already in use in phase I and II studies in humans [57], and thus this approach could soon become available for patients for various indications. It will potentially offer new avenues of functional preservation of the kidney, and at the same time help to further clarify the relevance of hypoxia in kidney disease.

Reprint requests to Kai-Uwe Eckardt, M.D., Medical Clinic 4, Krankenhausstrasse 12, 91054 Erlangen, Germany.
E-mail: kai-uwe.eckardt@med4.med.uni-erlangen.de

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